PRODUCTION OF POLYHYDROXYBUTYRATE (PHB) FROM *Bacillus cereus* BY USING RICE STRAW AS SUBSTRATE

NOR SYAFARAH BINTI ZAKARIYA

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Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

ABSTRACT

In this paper, there were 2 main objectives. Those were to To determine the potential of delignification rice straw by using *B.cereus* and to study the PHB production from *B.cereus* by using rice straw as substrate. Delignification was crutial due to its chemical durability makes it indigestible to organisms because of its bonding to cellulose and protein material. This lignin sheet acts as a barrier towards the outside elements (Carraher, 2008). The method used for delignification process was fermentation of *B.cereus* with 250 mL distilled water, 0.5 g yeast extract, 2.5 g peptone, 0.25 g $MgSO_4.7H_20$ and 10 g of rice straw in a 500 mL shake flask. Then, the bacteria were fermented in the medium at 30 ºC and 250 rpm. For the analysis of delignification of rice straw by *B.cereus*, Klason's method was utilized. From the result obtained, the highest lignin content was 4.56 % and the Control was 8.18 %. The percentage of the highest lignin degradation for the three samples was 98 %. The average lignin degradation was 78.67 %. After delignification, the bacteria could reacted on the cellulose content to synthesis PHB. The bacteria was fermented in 250 mL distilled water, 0.5 g yeast extract, 2.5 g peptone, 0.25 g $MgSO_4.7H_20$ and 1 g of delignified rice straw in a 500 mL shake flask. Then, the bacteria was fermented in the medium at 30 ºC and 250 rpm. The method of analysis of PHB yield was by using UV-vi spectrophotometer at 238nm. From the results obtained, the highest PHB yield is produced in Medium B; the medium consisted of $MgSO_4.7H_2O$ and yeast that yield 2.62 %. By these results, the objectives were achieved.

ABSTRAK

Terdapat dua objektif utama kajian ini iaitu untuk degradasi lignin dari jerami padi dan untuk menghasilkan PHB kedua-duanya daripada *B.cereus*. Pendeligninan penting kerana struktur kimianya yang menghubungkan selulosa dengan protein serta kompleks menyebabkannya sukar untuk dicernakan oleh organisma. Lignin bertindak sebagai penghalang terhadap element luar (Carraher, 2008). Proses yang digunakan untuk degradasi lignin ialah dengan menapaikan bakteria ke dalam media yang mengandungi 250 mL air suling, 0.5 g ekstrak yis, 2.5 g pepton, 0.25 g $MgSO_4.7H_20$ dan 10 g jerami padi di dalam 500 mL kelalang kon. Kemudian, bakteria dibiarkan dalam keadaan 30 ºC dan 250 rpm. Untuk menganalisa degradasi lignin, 'Klason's method' digunapakai. Berdasarkan kajian, peratus lignin tertinggi ialah 4.56 % dan Kawalan ialah 8.18 %. Manakala peratus degradasi lignin tertinggi 98 %. Manakala, purata delignifikasi ialah 78.67 %. Peratus delignifikasi ini menunjukkan nilai lignin yang berjaya dicernakan oleh bakteria. Selepas itu, proses seterusnya adalah pencernaan selulosa untuk menghasilkan PHB. Proses yang digunakan ialah dengan menapaikan bakteria ke dalam media yang mengandungi 250 mL air suling, 0.5 g ekstrak yis, 2.5 g pepton, 0.25 g $MgSO_4.7H_20$ dan 1 g jerami padi yang telah didegradasi lignin di dalam 500 mL kelalang kon. Untuk menganalisa penghasilan PHB, 'UV-vis spectrophotometer' digunakan pada 238 nm. Daripada hasil kajian, penghasilan PHB tertinggi ialah 2.62 % di dalam 'Medium B' iaitu media yang mengandungi $MgSO_4.7H_2O$ dan yis.

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LIST OF SYMBOLS/ABBREVIATIONS

ATP	= adenosine 5'-triphosphate
C	= carbon
Ca^{2+}	= calcium ion
COASH	= coenzyme A
Fe^{3+}	= ferum ion
g	= gram
hr	= hour
H_2O_2	= hydrogen peroxide
K^+	= potassium ion
kDa	= kilo Dalton
L	= liter
$MgSO_4.7H_2O$	= magnesium sulphate (hydrated)
mL	= mililiter
Mn^{2+} and Mn^{3+}	= manganese ion
MT	= metric ton
N	= nitrogen
NaCl	= sodium chloride
NAD^+	= nicotinamide adenine dinucleotide
NADPH	= nicotinamide adenine dinucleotide phosphate
nm	= nanometer
OD	= optical density
R	= carbon chain
rpm	= rotational per minute
S^+	= sulphur ion
µm	= micrometer
^oC	= degree Celcius

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The field of study for production of polyhydroxybutyrate (PHB) is revolve around polymer. Polymers are large molecules made up of repeating units of smaller molecules called monomers (Smith, 2006). Others included in the category polymers are important compounds such as proteins and carbohydrates. They also include such industrially important plastics such as polyethylene, poly(vinyl chloride) (PVC) and polystrene (Smith, 2006). Polymers that are biodegradable and made up entirely from biological products are called biopolymers. Polyhydroxybutyrate is included as biopolymer. In research filed of biopolymers, it has growing popularity among scientist as well as engineers each and every day (Gross & Kalra, 2002). Kennedy and Sundquist (1993) also summarize the general introduction of biopolymers, technical overview of biopolymer field and description regarding researches on biopolymer in Europe, Japan and Unites States of America. In addition, Lenz (1995) has made a packed summarize on 27 polymer researches that has been conducted in Japan. These shows that biopolymer is a significant compound as an alternative towards contemporary plastic today, that is unbiodegradable.

As mentioned above, biodegradable biopolymer (BDP) is an alternative for the petroleum derived plastic (such as polyethylene). Some BDP can degrade within days or months. The biodegradability is determine by the molecular structure (M Flieger *et al.,* 2003). PHB has a unique molecular structure. Eventhough it has the

same characteristic as polyethylene (but it is brittle), it is biodegradable as it is derived from biological compound such as bacteria or fungi.

Many researches has been done to obtain PHB due to its characteristic as a thermoplastic. Many of the researches to obtain PHB has been conducted by using bacteria. One of the it has been done on *Pseudomonas* sp. that was isolated from Antarctic environment with high stress resistant (D. Ayub *et al.,* 2004). Bacteria has a system to survive in a nutrient starvation condition and tolerate exposure to multiple stress agents. While running the research, the scientists were screening the PHB producing strains in *Bacillus* genus within the antarctic samples by using the classic heating method. But, what they have found is *Pseudomonas* sp. 14-3 strain that can produce high yield of PHB. The environment in Antarctica is always pressurised organisms there. Thus *Pseudomonas* sp. can produce high yield of PHB. Thus, this research contributes to the world of BDP greatly.

Besides *Pseudomonas* sp., other bacterias that has been used are those in activated sludge. A research has been done on synthesis of PHB from activated sludge by W.F.Hu *et al.* (1997). In the activated sludge, bacteria that can be isolated are *Alcaligenes* spp., *Pseudomonas* spp., recombinant *Escherichia coli* and a number of filamentous genera. This mixed culture were able to accumulate polyhydroxyalkanoates (PHA) and their copolymers as secondary metabolite or intracellular carbon reserve when unfavourable environmental conditions are encountered. This research contributes by proving that bacteria that is easily to be obtained can produce BDP and it can lower the cost of production of it. Another research is optimization of PHB production by *Bacillus* sp. CFR 256 strain with corn steep liquor (CSL) as its nitrogen source (S. V. N. Vijayendra *et al.,* 2007). The objective of the study was to economize the PHB production by optimizing the fermentation medium using steep liquor (by-product of starch processing industry) as a cheap nitrogen source by *Bacillus* sp. CFR 256. From the results obtained, the maximum PHB yields were found at highest CSL concentration (25 g/L). Even by using the cheap CSL, yield of PHB can be optimized. This research is important to reduce the production cost of BDP.

A state of the art technology is currently being research. Azuyuki Shimizu (2002) was doing a review on metabolic pathway analysis with emphasis on isotope labeling approach. The objective of this research is to treat metabolism as a network or entirely instead of individual reactions. The bacteria applied for PHB production are *Ralstonia eutropha* and recombinant *Escherichia coli*. At the end of the research, investigation on metabolic flux analysis with gene and protein expressions to uncover the metabolic regulation in relation to genetic modification or change in the culture condition. The research contributes in determine the optimum metabolic pathway to produce PHB and by genetically modify the bacteria, only PHB pathways will be triggered thus no or negligible by-product will be produced.

BDP is superior not by just on its own, but it is highly functional when mixed together with other substance. A very novel study that could be importance for the future is that of functionalized cellulose nanofibers and nanocrystals blended with biodegradable polymers and arclylic acid polymers. This is due to the nanocrystals were found to be markedly superior reinforcing agents than wood flood flour. Their behaviour is also similar to the exfoliated clays in terms of reinforcing properties (Varma, 2005).

1.2 Objectives

The objectives of this study are:

1. To determine the potential of delignification rice straw by using *B.cereus*
2. To study the PHB production from *B.cereus* by using rice straw as substrate

1.3 Scopes of Study

By using the objectives as basis, the scope of study are as follows:

1. Delignification of rice straw by *B.cereus* at optimum condition of pH 7, 30 ºC and 250 rpm.
2. PHB production by *B.cereus* at optimum condition of pH 7, 30 ºC and 250 rpm while the variables are the medium content of yeast, peptone and $MgSO_4.7H_2O$.

1.4 Problem Statement

Within the last few decades, plastic has revolutionized our lifes. Nowadays, plastics are everywhere, almost a must in everyday life. Most plastics are made of polyethylene chain of monomer, methylene (CH_2). The chain is commonly as long as from 1000 to 10000 of repeated CH_2. Due its long chain, hence it is spoken of as high polymers or macromolecules (J. Brydson, 1999). Its molecular structure contributes to unbiodegradability of it. In soil, plastic will takes up to 1000 years to degrade. Whereas in water, it will take 450 years to degrade. 260 million tonnes of plastic are used globally annually, accounting approximately 8 % of world oil production (Thompson *et al.,* 2009). Eventhough plastic usage are inexhaustible, most of it will be disposed off within a year or so after the manufacture (Barnes *et al.,* 2009). This will increase the solid waste from plastic.

Due to its nonbiodegradable property, it can endangered the wild life, marine life especially. Plastics in the ocean are called marine debris. Within 20 years since 1971, the total fish caught by the fishermen reduced by 90 %. The most obvious dwindled is the deep ocean species (Journal Fisheries Research, 2006). It is assumed that pelata fish will be instinct by the observations that showed that on 2005 and 2006, 1 tonne of the fish is caught. But, on 2000, the fish caught was 1621 tonne (Malaysian Inshore Fishermen Action Network, 2009). Marine debris ahave affected at least 267 species world wide, this includes 86 % of all sea turtle species, 44 % of

all sea bird species and 43 % of marine mammal species (Laist, 2997). Those are affected by ingestion, starvation, suffocation and entanglement. In 1980s, researches estimated that 100000 marine mammal deaths annually in the North Pacific related to entanglement in plastic nets and fishing line (Wallace, 1985). These proves that the plastic is affecting the marine lives. The plastic are looking edible thus they consume it. Polyethylene are not biodegradable, thus it will stay in their system. The toxicity in the polyethylene will consume the marine lives slowly and soon, they will die.

Apart from plastic, rice straw is also a liability. From 100 % of rice straw avalaibility after the harvesting season, 51 % from rice straw is thrown away by burning. This causes pollution. So, liability can be converted to assets. These assets are PHB that can contribute to two benefits; reduce air pollution by utilise the rice straw and produce BDP from PHB. Also, potential of availability of rice straw is very high because of the plantation of the rice will yield 0.45: 0.55 for rice straw with rice itself. This ratio were calculated by assuming that every hectare of paddy field will produce 4 to 5 tone of rice and that mean that it will produce almost as much as the rice amount. The collection or the harvesting of the paddy is usually made by the early of August to September and the second season is around December to February. Table 1.1 proves the abundant availability of rice straw in Malaysia.

Table 1.1 : The Number of Land Being Planted With Rice and the Rice Straw Being Produced Preseason.

Project (State)	**Area (Hectare)**	**Amount Of Rice Straw (MT)**
Kedah and Perlis	60,359	301,759
Kelantan	35,973	179,865
Perak	36,354	181,770
Penang	10,138	50,690
Selangor	8,500	42,500
Total	151,324	756,584

*The number of the rice straw amount were calculated by counting the are the rice plantation area. (Sources: MADA, KADA and BERNAMA website)

1.5 Rationale and Significance

The rationale of using PHB instead of plastic is that it has the same physical properties as plyethylene but only brittle yet biodegradable (M. Flieger, 2002). *Bacillus cereus* is used instead of fungi because fungi is easily to be spread through out the environment. This bacteria is also widespread in nature and isolated from soil and growing plants (Kramer, 1989) thus, it is easily obtained in rotting food or any food waste. This is also due to its hydrolytic activities on food components (T.S.M. Pirttija¨rvi, 2000). It is also easy to sporulate on most media easily after 2 to 3 days (Granum, 2007). This bacteria is also capable to utilize cellulose in aerobic and moderate temperature condition (Rajvaidya *et* al., 2006). Rice straw is used due its availability and abundant stock in Malaysia as well as its cellulose content (MADA).

CHAPTER 2

LITERATURE REVIEW

2.1 Polymer

2.1.1 Plastics

Most plastics are made of polyethylene chain of monomer, methylene (CH_2). The chain is commonly as long as from 1000 to 10000 of repeated CH_2. Due its long chain, hence it is spoken of as high polymers or macromolecules (J. Brydson, 1999). Its molecular structure contributes to unbiodegradability of it. In soil, plastic will takes up to 1000 years to degrade. Whereas in water, it will take 450 years to degrade. During 1930s, plastics had reached comercial status: poly(vinyl chloride), polystyrene, poly(methyl methacrylate), low density polyethylene and nylon (Pasquini, 2005).

Plastics are typically polymers of high molecular weight, and may contain other substances to improve performance or reduce costs (Wikipedia, 2008). The word derives from the Greek πλαστικός (plastikos), "fit for molding", from πλαστός (plastos) "molded". It refers to their malleability or plasticity during manufacture, that allow them to be cast, pressed, or extruded into an enormous variety of shapes such as films, fibers, plates, tubes, bottles, boxes, and much more. The most used plastic nowadays is polyethylene and polypropylene (Pasquini, 2005). Polypropylene is a type of polymer that was a branched of low molecular weight oil and it was discovered in 1950.

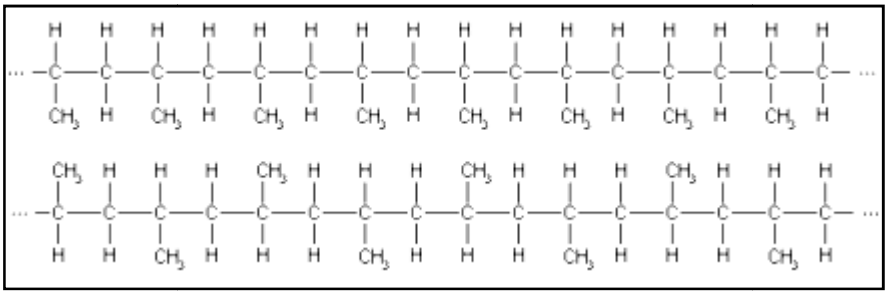

Figure 2.1 Molecular structure of polypropylene (Source: Absolute Astronomy, 2010)

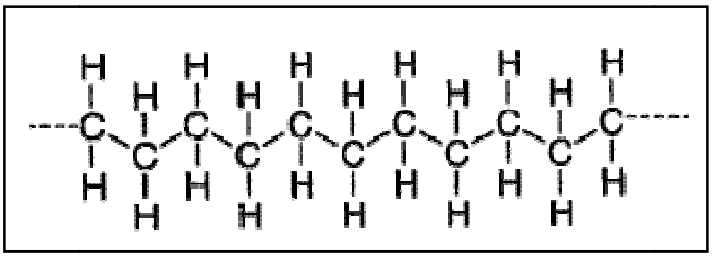

Figure 2.2 Molecular structure of polyethylene (Source: American Chemistry Council, 2010)

There are two types of plastic; thermoplastics and thermosets. Many of the polymers are thermoplastic. Thermoplastic is defined as it can be heated and reformed over and over again. This is important for easy processing and recycling. Unlike thermoplastic, thermosets cannot be reprocessed. If it is reheated, the material will scorch. Plastics can also be molded into bottles or anythong else as well as mixed with solvents to become an adhesive or paint. Plastics can deterioriate but can never decompose completely (American Chemistry Council, 2010)

Plastics are also applies to a wide range of materials that at some stage in manufacture are capable of flow such that they can be extruded, moulded, cast, spun or applied as a coating. Synthetic polymers are typically prepared by polymerization of monomers derived from oil or gas, and plastics are usually made from these by addition of various chemical additives. There are currently some 20 different groups of plastics, each with numerous grades and varieties. Plastics are incredibly versatile materials; they are inexpensive, lightweight, strong, durable, corrosion-resistant, with high thermal and electrical insulation properties. The diversity of polymers and the

versatility of their properties facilitate the production of a vast array of plastic products that bring technological advances, energy savings and numerous other societal benefits (Andrady & Neal 2009). The first truly synthetic polymer, *Bakelite*, was developed by Belgian chemist Leo Baekeland in 1907, and many other plastics were subsequently developed over the next few decades. It was not until the 1940s and 1950s, however, that mass production of everyday plastic items really commenced (Thompson, 2009)

2.1.2 Biopolymer

Plastic mentioned is a type of polymer, but synthetic polymer. It is because it is chemically derived from petroleum. Biopolymer are made biologically or naturally occured in the environment. Biopolymers are polymers that are generated from renewable natural sources, are often biodegradable and nontoxic. They can be produced by biological systems (such as microorganisms, plants and animals), or chemically synthesized from biological materials (such as sugars, starch, natural fats or oils) (Flieger *et al.*, 2002). To produce biopolymer chemically, it can be classified into three groups. Those are polyesters, polymers containing esther and other heteroatom-containing linkages in the main chain and also polyamino acids (Okada, 2002).

The common biopolymer in the environment is polysaccharide or known as starch. Starch is a major plant storage form of glucose. It consists of two components. Those are amylose, in which the glucose units are 1,4-α-D-linked together in straight chains, and amylopectin (can be identified by colored by elemental iodine) in which the glucose chains are highly branched. (Flieger *et al.,* 2002).

Table 2.1 : Some polysaccharides and their constituent monomers (Rastogi, 2003)

Polysaccharide	Monosaccharide units	Location and properties
Starch	D-glucose	Storage in plants
Mannans	D-Manose	Linear storage some higher plants
Cellulose	D-glucose	Structural polysaccharide in cell walls
Pectin	D-galactouronic acid	Fruits
Inulin	D-fructose	Linear polyfructosan in some plants
Glycogen	D-glucose	Branched storage polymer in animals

2.1.3 Polyhydroxybutyrate (PHB)

PHB is a type of biodegradable polymer (BDP), a family of polyhydroxyalkanoates (PHA) (Chen, 2005) and secondary metabolite (Rehm, 1997). Biodegradation is a process that breaks down a xenobiotic into simpler form. Ultimately, the biodegradation of organics results in the release of carbon dioxide (CO_2) and water (H_2O) to the environment (G. Landis *et al.*, 2003). The most commonly studied PHA, polyhydoxybutyrate (PHB), is known to possess physical properties similar to those of polyethylene, and has potential applications as disposable bulk material in packing films, containers, or paper coatings, amongst others (Quillaguamán *et al*., 2007).

PHB is an energy reserve polyester naturally accumulated by a wide variety of microbes. PHB-like copolymer is PHBV (polyhydroxybutyrate valerate). It is less stiff and tougher thus used as packaging material. Melting point of PHB is 40 -180 ºC. PHB has the same properties as polypropylene but stiffer and brittle. PHB also degradable in microbially active environments from 5 to 6 weeks (Shimao, 2001). The mode of action to be degraded is the enzyme from bacteria degrade the PHB.

Then it is absorbed through the cell wall and metabolized. If it is aerobic condition, it will be degraded to CO2 and H2O. But, in anaerobic condition, it is degraded to methane, CO2 and H2O (Lieger, 2002).

2.1.3.1 PHB Molecular Structure

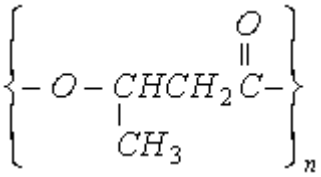

Figure 2.3 Molecular structure of PHB (Maia *et al.,* 2004)

PHB is the most common member of PHA. It belongs to to the short chain length PHA (scl PHA) with its monomers constitute of 4 to 5 carbon atoms. It is usually brittle but has a thermoplastic characteristic (Chen, 2005).

2.1.3.2 PHB as Secondary Metabolite

Secondary metabolite is the a byproduct synthesize by microorganism, not the major product and it increased greatly when substrates depleted from the medium (Rehm *et al.*, 1997). PHB is secondary metabolite because it is synthesize by microbes when there is no sufficent nutrient to promote their growth, but excess in carbon source. IT does not play essential role in development of the producing organisms, but convey advantages to the pertinent species concerning its long term survival in the biological community anda environment (Rehm *et al.*, 2997). Maximum production is when growth promoting substrates were depleted from the medium and this phenomenon is calle ‘catabolic regulation’ (Rehm *et al.*, 1997).

The causes of PHB may be produced as secondary metabolite are:

a) Nutritional downshift in the media caused by limitation of particular metabolites promote excessive formation of some metabolite due to an imbalanced metabolism. These accumulates or precursor is known to induce secondary pathways.
b) Limitation of some endegenous metabolites could be important which inhibit global regulatory mechanisms governing aerial mycelium and spore formation. Thus in this repressing or inhibitory effects on the secondary pathways and on morphogenesis could be diminished. (Rehm *et al.,* 1997)

2.1.3.3 Process Synthesis of PHB

PHB is synthesized by acetyl-CoA by the sequencial action of 3 enzymes; B-ketothiolase (phbA), acetyl-CoA reductase (phbB) and PHB polymerase (phbC). Production of PHB in plant is based on consumption of acetyl-CoA (initial substance). So, oil crops is a potential target for production of PHB due to its high flux of acetyl-CoA during the oil synthesis stage (Redd *et al.,* 2003; Omidvar *et al.,* 2008).

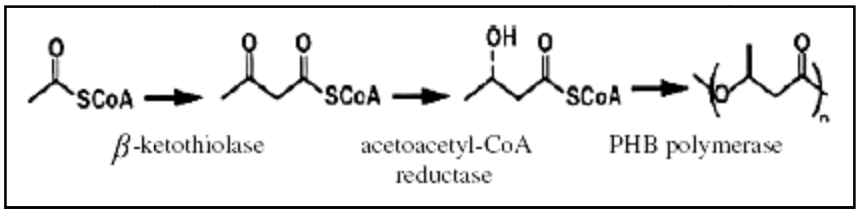

Figure 2.4 Polyhydroxybutyrate (PHB) biosynthetic pathway. PHB is synthesized by the sequential action of b-ketothiolase (phbA), acetoacetyl-CoA reductase (phbB), and PHB polymerase (phbC) in a three-step pathway (Madison and Huisman 1992)

To produce PHB, theoretical yields of PHB from several carbon sources have been estimated from biochemical pathways leading to PHB. In estimating the yields,

a special emphasis is made on recycling (or regeneration) of $NADP^+$ which is the co-substrate of acetoacetyl-CoA reductase, one of three key enzymes involved in the biosynthesis of PHB. As a $NADP^+$-regenerating enzyme, glucose-6-phosphate dehydrogenase or isocitrate dehydrogenase is conceived (Yamane, 1992). Figure 2.4 illustrates how PHB can be synthesized by metabolism of bacteria: The pathway to produce PHB is as in Figure 2.5.

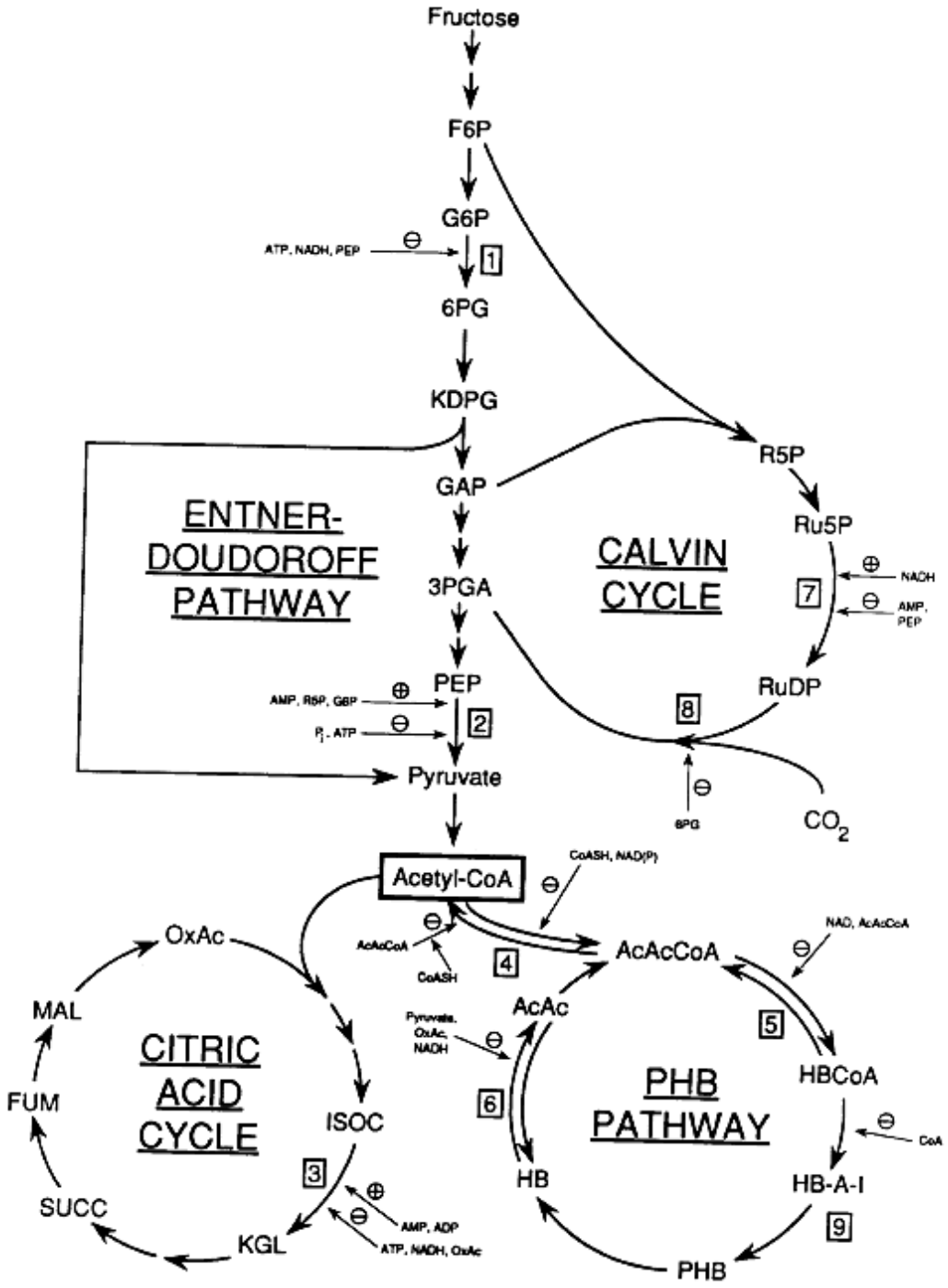

Figure 2.5 Metabolic pathway to produce PHB (Guske, 1990)

2.2 Carbon Source

Carbon source that is used in this paper is cellulose from rice straw. *B.cereus* will utilise cellulose for energy and growth. This is due to the fact that the bacteria needs carbon source to continue its system. Rice straw is used as second generation energy source instead of using first generation due to humanity issues (such as starvation).

So, before proceeding to the experiments, cellulose content and its availability in rice straw is investegated. Comparison between the content in the rice straw and other wastes is also observed.

2.2.1 Rice Straw

The chemical composition in rice straw plays the major role as a substrate for PHB production. In rice straw, there is not only cellulose available, but also contains other carbon based elements. One of the major carbon elements is hemicellulose.

Table 2.2 summarizes the contents of cellulose and hemicellulose available in the rice rice straw:

Table 2.2 : Carbon source content in rice straw (Zhu *et al.,* 2005)

Carbon source	Content (%)
Cellulose	38.6
Hemicellulose	19.7

Whereas Table 2.3 is the list of chemical properties contain in three types of plant wastes: rice straw, rice husk and wheat straw to highlight the particulate differences in feedstock as below:

Table 2.3 : Proximate composition and selected major elements of ash in rice straw, rice husk and wheat straw (Jenkins *et al.,* 1998)

	Rice straw	**Rice husk**	**Wheat straw**
Proximate analysis (% dry fuel)			
Fixed carbon	15.86	16.22	17.71
Volatile matter	65.47	63.52	75.27
Ash	18.67	20.26	7.02
Total	100.00	100.00	100.00
Element composition oof ash (%)			
SiO_2	74.67	91.42	55.32
CaO	3.01	3.21	6.14
MgO	1.75	<0.01	1.06
Na_2O	0.96	0.21	1.71
K_2O	12.30	3.71	25.60

Rice straw feedstock has low total alkali content (Na_2O and K_2O comprise < 15% of total ash) whereas wheat straws have < 25 alkali content in ash (Baxter et al., 1996). Rice husk which is also of poor feed quality, caused mainly by high silica. The fixed carbon shows that rice straw has 15.86 % of cellulose and other major carbon substance.

2.2.2 Carbon Source: Cellulose

Plant biomass consists of three main polymeric components. Those are cellulose, hemicellulose and lignin. In softwoods, hardwoods and the abundantly available agricultural residues such as wheat, rice and other cereal straws, sugarcane bagasse, corn stalks, corncobs, jute and cotton stalks, cellulose is the chief constituent (over 40 % by weight), then hemicellulose (~ 30 %) and lignin (~ 20%).

This shows that cellulose is the most abundant compound on earth, followed by hemicellulose and lignin (Varma, 2005).

Cellulose is very resitant to hydrolysis. This is due to the straight chain of B-1,4-linked glucose units without any side chains. Thus, extensive hydrogen bonding between the cellulose molecules that forms crystalline structure. At least, three different enzymes are required for the complete hydrolysis of the crystalline. Those are endo-ß-glucanase, exo-ß-glucanase and cellobiase (Kim, 2008).

2.2.2.1 Molecular Structure of Cellulose

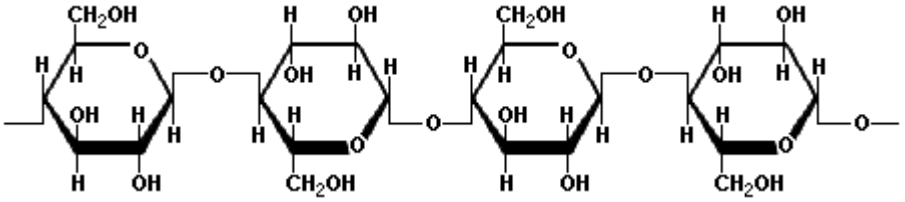

Figure 2.6 Molecular structure of cellulose (Sientific Psychic, 2005)

It is the major structural compound of cell wall of higher plants. Cellulose is also a high molecular weight linear polysaccharides of D-glucopyranose monomer units that joined together by B-1→ 4 linkage or also known as B-(1→4) glucosidic linkage. The B(1→4) adopt a fully extended conformation in which the glucose units zig zag along the polymeric chain (Tsai, 2007). Pickering (2000) also stated that cellulose cell wall provides structural support. This is due to the pressure of cell contents leads to turgidity thus may break the cell wall if no cellulose present. Osmotic intake of water can can damage the cell. Thus, cellulose is important to protect it and made the cell wall permeable to water and other dissolved substances.

According to Rastogi (2003), monomer of cellulose is D-glucose and in the plant cell wall, it is associated with lignin. Cellulose may be hydrolysed to its monomers. The process is called hydrolysis. There are two types of hydrolysis

process. Those are chemical and biological hydrolysis. For biological hydrolysis, the hydrolyse element is the reaction of the enzyme from the bacteria (such as cellulase). Whereas chemical hydrolysis uses two ways two do the actions. They are dilute acid hydrolysis or concentrated acid hyrolysis. Strong acid that is commonly use due to its high conversion is sulfphuric acid. Diluted acid hydrolysis is using diluted as hydrolyser (Rastogi, 2003). These reactions has different products eventhough the objective is the same. The summary of the reaction is:

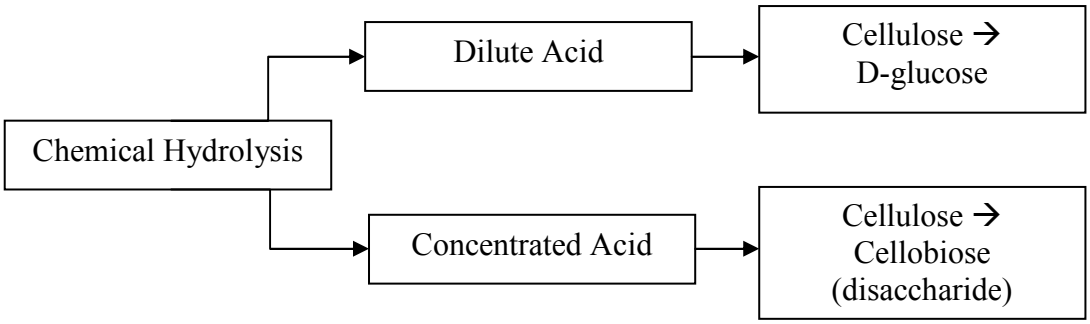

Figure 2.7 Summary reaction of chemical hydrolysis (Rastogi, 2003)

2.2.2.2 Degradation of Cellulose by Bacteria

Three enzymes react on degradation of cellulose are C_1, Glucanase or Cx and β-glucosidase. (Rajvaidya *et al*., 2006)

C1 acts on native cellulose, then glucanase cleave the partially degraded cellulose. Thus, cellobiose and oligamers are formed. Glucanase claeve the bonds between glucose units at random. Lastly, β-glucosidase hydrolyze the cellobiose and oligomers into glucose. The regulation of this process is regulated by a regulatory mechanism called catabolic repression (Rajvaidya *et al*., 2006).